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Analytical Method Validation as the First Step in Drug Quality Control

Sigrid Mennickent and Marta de Diego

Abstract

The authors have developed and validated some chromatographic methods with the aim of quantifying drugs as drug substance and drug product, suitable for stability and quality control studies, as at original products as at its remainder doses. The stability of a pharmaceutical is defined by its resistance to different chemical, physical, and microbiological reactions that may change their original properties. The stability of a pharmaceutical product is closely related to its potency; therefore, whether the compounds are degraded, a decrease of the therapeutic effect or changes in their toxicological properties can be produced, affecting their efficacy and safety, which becomes important to maintain a stable pharmaceutical product and to have the analytical tools to demonstrate stability. Therefore, stability-indicating methods are required to the quality control of pharmaceuticals. Analytical methods presented here are useful stability-indicating methods to analyze drugs and have adequate linearity, precision, accuracy, selectivity, and LOD/LOQ values. The examples presented here are stability-indicating methods since they allow the determination of drugs in the presence of their degradation products, according to the International Conference on Harmonization (ICH) guidelines.

Keywords: drug stability, stress testing, method validation, stability-indicating methods, drugs

1. Introduction

1.1 Drug stability

Pharmaceutical stability is the capability of a dosage form included in a specific container to suffer minimum or no degradation during its transport, storage, and use. It can be influenced by intrinsic factors such as the chemical structure of the drug and environmental conditions, as temperature, humidity, oxygen, and light. Each ingredient, whether therapeutically active or pharmaceutically necessary (excipients), can affect the stability. The primary environmental factors that can reduce stability include exposure to adverse temperatures, light, humidity, oxygen, and carbon dioxide. Various types of reactions can cause chemical degradation of pharmaceuticals, which usually cause loss of active drug content and formation of degradation products. These reactions are hydrolysis, oxidation, photolysis, dehydration, isomerization, polymerization, decarboxylation, absorption of carbon

dioxide, and radiation-induced reactions. The most common reactions are hydrolysis and oxidation. Chemical degradation may result in a loss of potency or an increase in drug toxicity, so that the clinical use of a medicine must be unacceptable if the degradation is relatively great. When a drug dosage form is altered (by dissolution, pulverization, or addition to other materials) or the environment of the drug is modified by changes in storage conditions, the stability of a drug may be affected [1–4]. Stability is often expressed in quantitative terms as the shelf life, that is, the time from manufacture to the original potency or content of active constituent has been reduced by 10%. For most products, this 10% limit for chemical degradation is generally recognized as the minimum acceptable potency level [5].

Stability testing forms an important part of the process of drug development; its purpose is to provide evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factors, such as humidity, temperature, and light, to establish a retest period for the drug substance or a shelf life for the drug product, and to recommended storage conditions. The studies are designed to include testing of attributes susceptible to change during storage and are likely to influence quality, safety, and efficacy.

Testing primarily covers physical, chemical, and microbiological attributes:

- Physical: appearance, melting point, water content, clarity and color of solution, pH, dissolution and disintegration characteristics, viscosity, crystal modification, or particle size
- Chemical: assay, degradation products, related substances, and residual solvents
- Microbial: growth in microorganisms and efficiency of preservative contents such as antioxidants and antimicrobial preservatives

Stability studies on active substances and manufactured dosage forms are conducted by means of accelerated, long-term studies and stress testing. Accelerated and long-term tests are developed at specific temperatures and relative humidity representing storage conditions experienced in the distribution chain of the climatic zone(s) of the country or region of the world concerned. The aim of these studies is to determine the shelf life of the product. Stress studies are conducted to elucidate the intrinsic stability of the drug substance and are normally carried out under more severe conditions than those used for accelerated testing. Stress testing is the main tool that is used to predict stability problems, with the aim to anticipate the behavior of the drug substance when using it as a drug product [6, 7]. From the results of stress testing, useful information can be obtained for the manufacturing process, packaging development, and appropriate storage conditions of the products in order to avoid degradation [7]. The chemical stability is evaluated by testing the quantity of drug at different times during the storage [2, 3].

1.2 Pharmaceutical quality control/pharmaceutical assurance

The term drug quality control refers to the sum of all procedures undertaken to ensure the identity and purity of a particular pharmaceutical, as active ingredient and dosage forms. These procedures involve the identification of a pharmaceutical substance; potency, usually 90–110% of the labeled amount; uniformity of color; shape; size in dosage forms; bioavailability; and stability, in concordance with the requirements of pharmacopoeial monographs (International Pharmacopeia,

European Pharmacopeia, US Pharmacopeia, British Pharmacopeia, National Pharmacopeia), and it was carried out in quality control laboratories [8, 9].

Quality control (QC) is an essential operation of the pharmaceutical industry to guarantee a safe and effective product. QC measurements include stability testing of the drug formulation, dissolution testing, and analysis of raw material products majorly.

QC is the term usually employed; however, there are two terms about drug or pharmaceutical quality control:

1.2.1 Pharmaceutical quality assurance (QA)

Quality assurance involves the development and implementation of a system that ensures that the pharmaceutical is safe; effective, with standard quality; and acceptable to the patient. It is achieved with systematic activities implemented in a quality system to ensure that the requirements for product development are fulfilled and involve appropriate storage, distribution, monitoring and use by prescribers, dispensers and consumers.

1.2.2 Pharmaceutical quality control (QC)

All of activities are required to control the processes associated with the product manufacture and evaluation of product quality at various steps from raw materials to the final packaged product that reaches the consumer.

In this way, good manufacturing practices (GMPs) are part of the quality assurance activities that ensure that products are consistently produced and controlled to the quality standards appropriate to their intended use and required by the drug regulatory authorities, for example, personnel, facilities, packaging, and quality control.

Assuring quality involves some aspects, as:

- Active ingredients are safe and efficient
- Providers have acceptable quality standards
- Active ingredients and pharmaceutical in dosage forms are monitored to meet quality standards
- Pharmaceutical packaging is optimal and protects from degradation
- Storage conditions of pharmaceutical are optimal
- Transportation conditions are adequate
- Product quality concerns are reported and monitored

Low quality medicines can produce a decrease of their therapeutic effect or changes in their toxicological properties, which can cause a prolongation of illness and even death, and an increase in health costs.

1.3 Stability testing for drug substances and drug products (pharmaceuticals)

Stability testing predicts behavior of a drug or pharmaceutical with any physical, chemical, or microbiological changes, with the aim to assess their security and efficacy and to establish their shelf life and optimal storage conditions [10–21].

1.4 Stress testing (forced degradation studies)

Stress testing is an important part of drug development process because it can help to establish the degradation pathways and the intrinsic stability of the molecule and help to develop stability-indicating methods. Stress testing helps to anticipate the behavior of drug substance and drug products and their mechanisms (hydrolysis, oxidation, thermolysis, or photolysis) and identify the degradation products, including their chemical structure. These studies are a regulatory requirement and scientific necessity during drug development, with the aim to generate more stable formulations. Stress testing includes the effect of temperatures and other appropriate conditions such as humidity, light exposition, and others. Because it is faster and less expensive than conducting longer-term storage tests, the technique is used for rapid selection and elimination tests. The samples generated from forced degradation can be used to develop the stability-indicating method that can be applied later for the analysis of samples generated from accelerated and long-term stability studies [1–4, 22].

The nature of the stress testing depends on the individual drug substance and the type of pharmaceutical product involved. Stress testing is likely to be carried out on a single batch of the active pharmaceutical ingredient. Generally, the goal of stress testing is to facilitate an approximate 5–20% degradation of the sample under any given condition, so as to avoid any secondary reactions. It should include the effect of temperature, above that for accelerated testing (in 10°C increments, e.g., 50°C, 60°C, etc.), humidity (75% RH or greater), oxidation, photolysis, and the susceptibility to hydrolysis on the active pharmaceutical ingredient (API) [7, 23].

Stress testing is likely to be carried out on a single batch of the active pharmaceutical ingredient. It should include the effect of temperature (in 10°C increments, e.g., 50°C, 60°C) above that for accelerated testing, humidity (25°C, 75% RH or greater) where appropriate, oxidation, and photolysis on the active pharmaceutical ingredient (API). The testing must also evaluate the susceptibility of API to hydrolysis and its photostability. Significant change for a drug substance is defined as failure to meet its specification [24].

2. Analytical method validation as the first step in drug quality control

To evaluate the stability of drug substances or drug product, qualitative and quantitative methods should be used, to allow evaluate the physical, chemical, biological and microbiological stability.

In relation to chemical stability, the assay methods chosen should be those indicative of stability. These methods will resolve all degradation products from the parent compounds and ideally from each other, so that the active compound content can be accurately measured without interference from degradation products [7]. The most used analytical method in drug quality control is still high-performance liquid chromatography (HPLC) coupled with UV or fluorescence detection. Other useful methods are LC coupled with mass spectrometry (MS) or with diode array (LC/DAD), refractive index (RI), electrochemical, light-scattering detection, size exclusion chromatography (SEC), UPLC, capillary electrophoresis (CE), and gas chromatography (GC). High-performance thin-layer chromatography (HPTLC) has the advantages of its fastness, solvent economy, and high throughput of samples. Chromatography of samples simultaneously with standards is another advantage, allowing to run up to 60 spots simultaneously. Sensitivity of HPTLC is normally in the range of nanograms in absorbance and picograms in fluorescence mode (www.europeanpharmaceuticalreview.com.Access: 30-10-2018; [25]).

2.1 Importance of analytical method validation used in drug quality control

Analytical method validation is an integral part of any good analytical practice. The results from a method validation procedure can be used to judge the quality, reliability, and consistency of analytical results. Analytical methods should be validated or verified, and the accuracy as well as the precision (standard deviations), limit of detection (LOD) and limit of quantification (LOQ), sensitivity, linearity, and applicability should be recorded. The tests for related compounds or products of decomposition should be validated to demonstrate that they are specific to the product being examined and are of adequate sensitivity. Validated analytical methods play a major role in achieving the quality and safety of the final product especially in the pharmaceutical industry [26]. Among the FDA, some other useful protocols can be addressed by the International Council on Harmonization (ICH), current good manufacturing practice (CGMP), US Pharmacopeia (USP), Turkish Pharmacopeia, European Medicines Agency (EMA), International Organization for Standardization (ISO), Association of Analytical Chemists (AOAC), and the American Public Health Association [3, 6, 24, 26, 27–31].

2.2 Some stability-indicating methods developed and validated by authors

The authors have developed and validated some chromatographic methods suitable for quality control studies of bulk drugs and drugs in dosage forms.

2.3 Chemical stability of haloperidol injection by high-performance thin-layer chromatography

Enalapril is an angiotensin-converting enzyme (ACE) inhibitor. Enalapril (**Figure 1**) is a prodrug and has little pharmacological activity until hydrolyzed in the liver to enalaprilat (**Figure 2**). Enalapril is the ethyl ester of enalaprilat [1, 4, 32–35].

Enalapril is used alone or in combination with other classes of antihypertensive agents for the management of hypertension. It is reported that enalapril drug substance or pharmaceutical preparation degrades to two major degradation products, enalaprilat and diketopiperazine (DKP) derivative, under different storage conditions, resulting in drug loss and potency reduction [1, 4, 32–35].

A stability-indicating high-performance thin-layer chromatographic (HPTLC) method was developed and validated by the authors, with the aim of determination of enalapril maleate in tablets.

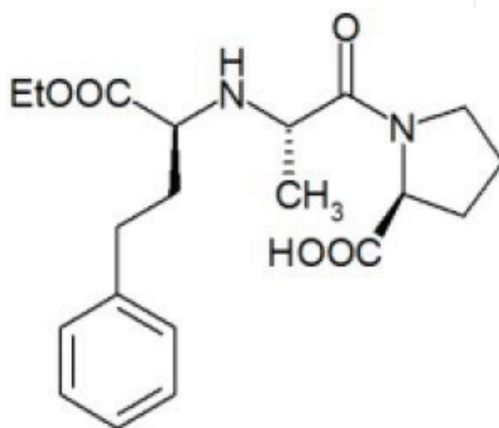


Figure 1.
Chemical structure of enalapril.

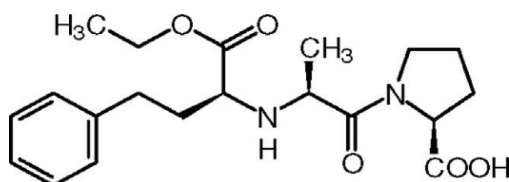


Figure 2.
Chemical structure of enalaprilat.

Chromatographic separation was achieved on precoated silica gel F 254 HPTLC plates using a mixture of 1-butanol, glacial acetic acid, and water (12:3:5, v/v) as a mobile phase. Quantitative analysis was carried out at a wavelength of 207 nm. The method exhibited an adequate linearity with a correlation coefficient of 0.998, over the concentrations range of 200 to 1200 ng/ μ L (200 to 1200 ng/band). Limit of detection (LOD) was 23.78 ng/band, and limit of quantification (LOQ) was 72.01 ng/band. The method exhibited a good precision, with an intra-assay variation between 1.14 and 1.43% and an inter-assay variation between 1.27 and 3.67%; an adequate accuracy, good selectivity (R_f was 0.52 for enalaprilat, 0.62 for enalapril, and 0.82 for diketopiperazine (DKP), a degradation product. Also, the selectivity between enalapril and hydrochlorothiazide, the more common compounds in the commercial mixtures of enalapril, was studied. The R_f was 0.52 for enalaprilat, 0.62 for enalapril, and 0.83 for hydrochlorothiazide (**Figures 3 and 4**) [36].

Stability-indicating capability of the HPTLC assay was studied by forced decomposition of 5 mL of a solution of enalapril 1 mg/mL, with 10 mL of 0.1 N hydrochloric acid, 10 mL of 0.1 N sodium hydroxide, and 10 mL of 3% H_2O_2 . The mixtures

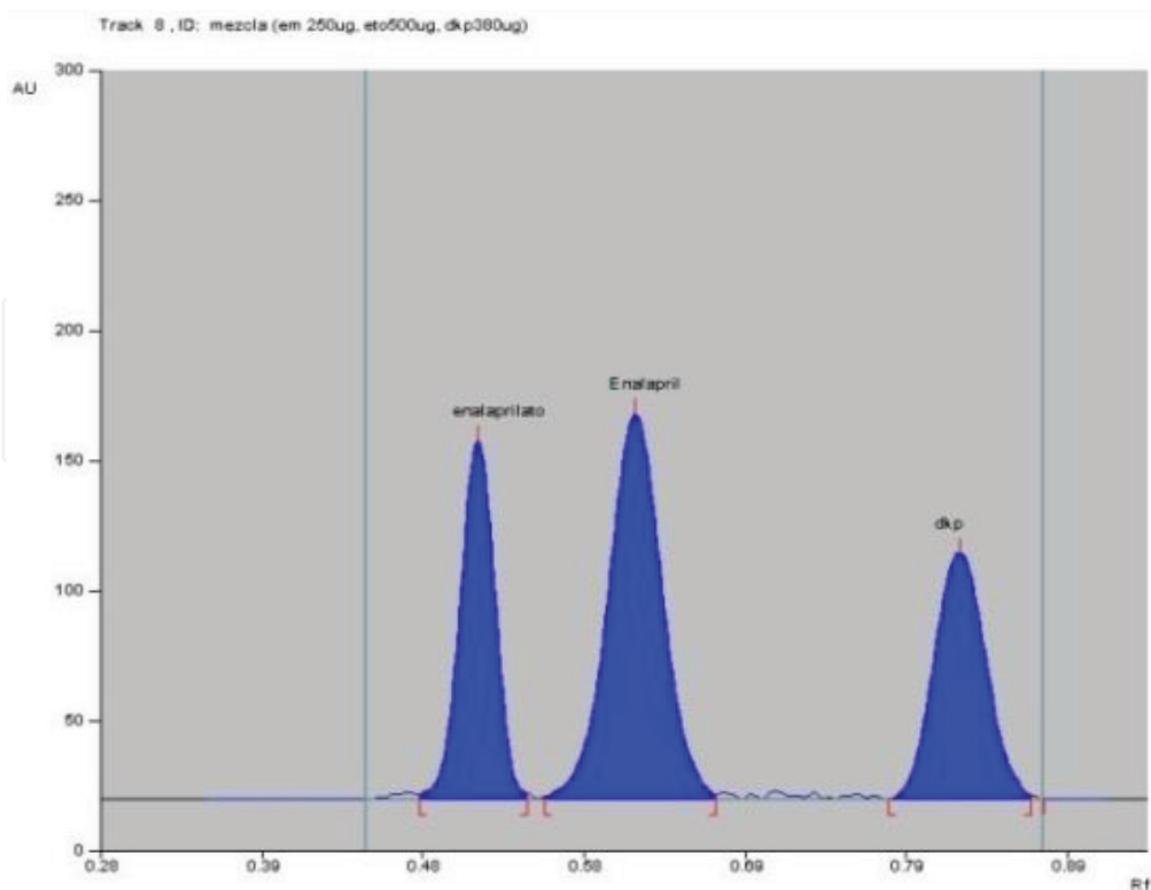


Figure 3.
Selectivity of the method. A study with enalapril, enalaprilat, and DKP. Peak observed: enalaprilat, enalapril, and DKP. R_f , retarding factor; AU, absorbance unit.

with NaOH and with HCl were heated on hot plates at 60°C for 60 min. The mixture with H₂O₂ was stored at room temperature (25°C) for 60 min. Then, each mixture was diluted with ethanol to 100 ng/μL and analyzed. Also, stability-indicating capability of the assay was proven by conducting forced degradation conditions of UV and VIS radiation on enalapril standard, as solution of 100 ng/μL. Degradation was found significantly in basic stress condition only, during the time of the study. The degradation products for enalapril mentioned in the literature are enalaprilat and DKP [34, 36]. The degradation product was well resolved from the main peak, proving the stability-indicating power of the method (Figure 5) [37].

2.4 Validated instrumental planar chromatographic method for quantification of fluphenazine hydrochloride in injections

Fluphenazine (Figure 6) is a phenothiazine antipsychotic agent. The drug is a propylpiperazine derivative of phenothiazine and is structurally similar to perphenazine but differs from perphenazine in the substitution of a trifluoromethyl group for chlorine at the two positions of the phenothiazine nucleus.

Drug therapy with fluphenazine is integral for the management of acute psychotic episodes with violent behavior in patients with schizophrenia and generally is required for long-term stabilization to improve symptoms between episodes and to minimize the risk of recurrent acute episodes.

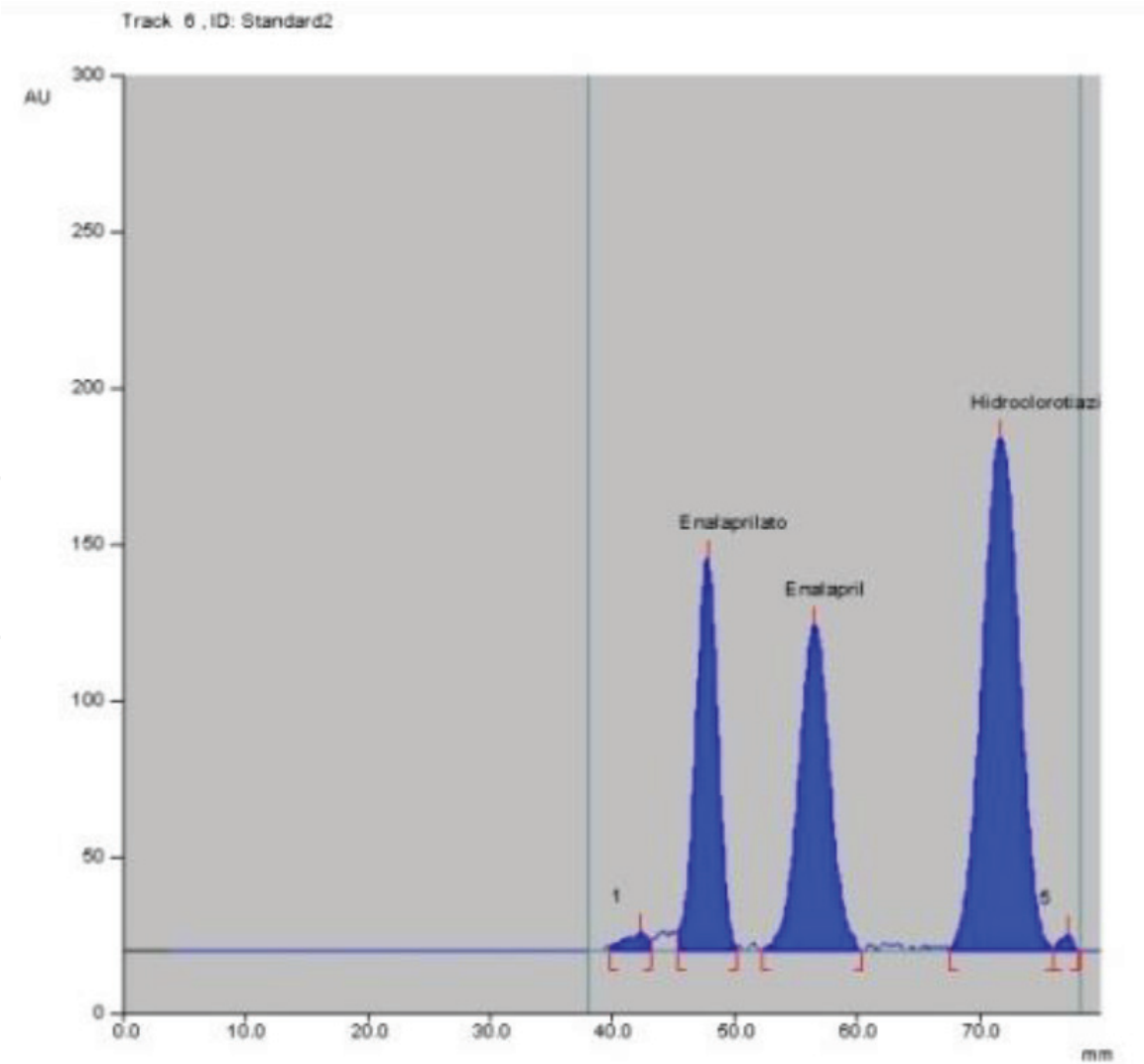


Figure 4. Selectivity of the method. A study with enalapril, enalaprilat, and hydrochlorothiazide. Peak observed: enalaprilat, enalapril, and hydrochlorothiazide. R_f, retarding factor; AU, absorbance unit.

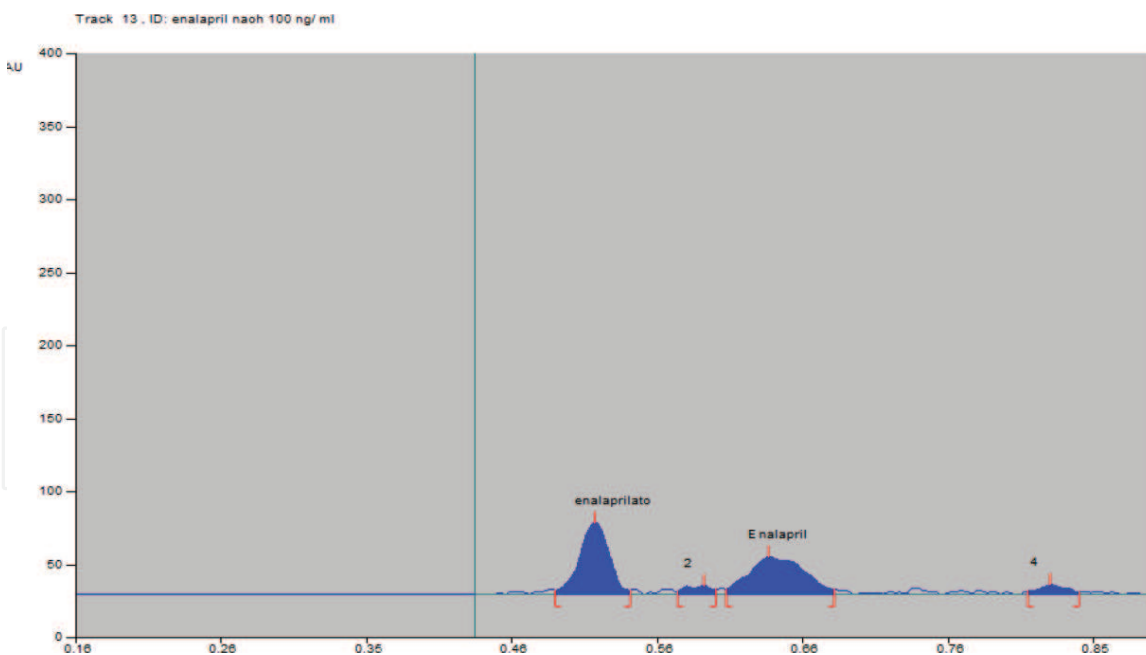


Figure 5.

A study at forced degradation of enalapril with 0.1 N NaOH. Peak no. 1, enalaprilato; peak no. 2, enalapril; and peak no. 3, DKP. Axis X: retardation factor (Rf). Axis Y: absorbance unit (AU).

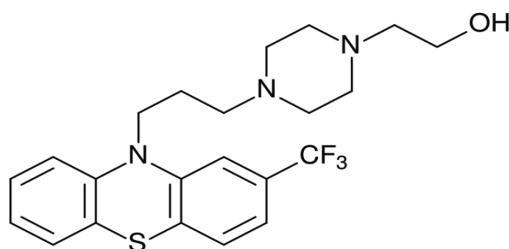


Figure 6.

Chemical structure of fluphenazine.

Other uses for fluphenazine are for the treatment of mania, bipolar disorder, severe anxiety, and behavioral disturbances. Fluphenazine is administered as the hydrochloride by mouth or intramuscular injection; longer-acting decanoate or enantate esters of fluphenazine are given by intramuscular or sometimes subcutaneous depot injection [32, 35, 43].

Fluphenazine hydrochloride, decanoate, and enantate are all sensitive to light. Therefore, it is very important to determine the quantity of fluphenazine in its dosage forms because in the presence of light, photolysis occurs rapidly, resulting in drug loss and potency reduction [2, 4, 32–35].

Chromatographic separation was done on precoated silica gel F 254 HPTLC plates. The mobile phase consisted of methanol/purified water (9:1, v/v). Densitometric analysis was carried out at 306 nm. The calibration curves were linear in the range of 100 ng/μL to 500 ng/band with a correlation coefficient of 0.998. The limit of detection (LOD) and limit of quantification (LOQ) were found to be 1.45 ng and 4.40 ng, respectively. The intra-assay and inter-assay precisions, expressed as the relative standard deviation (RSD), were in the range of 0.73–1.77% and 1.18–1.86%, respectively. The recovery of fluphenazine hydrochloride was between 98.29 and 101.53%, with a RSD not higher than 1.87%. The method was selective for fluphenazine hydrochloride from the preservatives of the injections (Rf for fluphenazine hydrochloride was 0.33, whereas parabens run to the solvent front) (Figures 7 and 8) [38].

Drug content was found to be within the limits (95–110% of the labeled content of the formulations) of the prescribed value, when the method was applied to quantify fluphenazine hydrochloride in real pharmaceutical samples.

Stability-indicating capability of the HPTLC assay was studied by forced decomposition of 5 mL of a solution of fluphenazine 1 mg/mL, with 10 mL of 0.1 N hydrochloric acid, 10 mL of 0.1 N sodium hydroxide, and 10 mL of 3% H₂O₂. The mixtures with NaOH and with HCl were heated on hot plates at 60°C for 60 min. The mixture with H₂O₂ was stored at room temperature (25°C) for 60 min. Then, each mixture was diluted with ethanol to 100 ng/μL and analyzed.

Also, stability-indicating capability of the assay was proven by conducting forced degradation conditions of UV and VIS radiation on fluphenazine standard, as solution of 100 ng/μL.

One degradation product was found after treatment of fluphenazine with HCl, and two degradation products were found after treatment with NaOH. R_f for fluphenazine was 0.30, whereas R_f for degradation product with HCl was 0.01, and R_f for degradation products with NaOH were 0.03 and, 0.23 respectively (**Figure 9**). None degradation product was found with H₂O₂.

When the drug was exposed to force degradation with VIS radiation, another peak different from the peak of fluphenazine was found; therefore, it could be a degradation

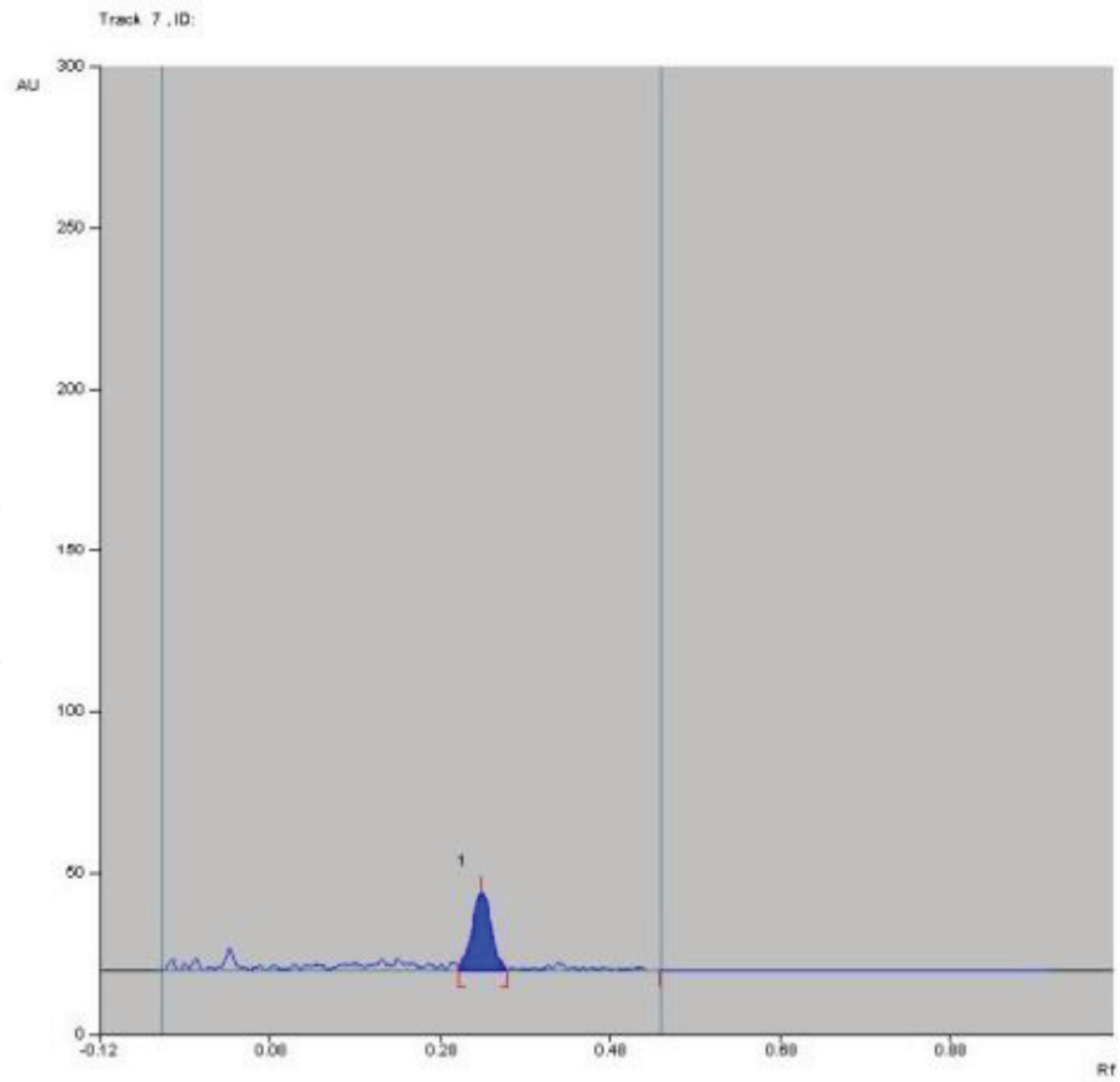


Figure 7.
Selectivity of the method. A study with parabens. Peak observed: fluphenazine. R_f, retarding factor; AU, absorbance unit.

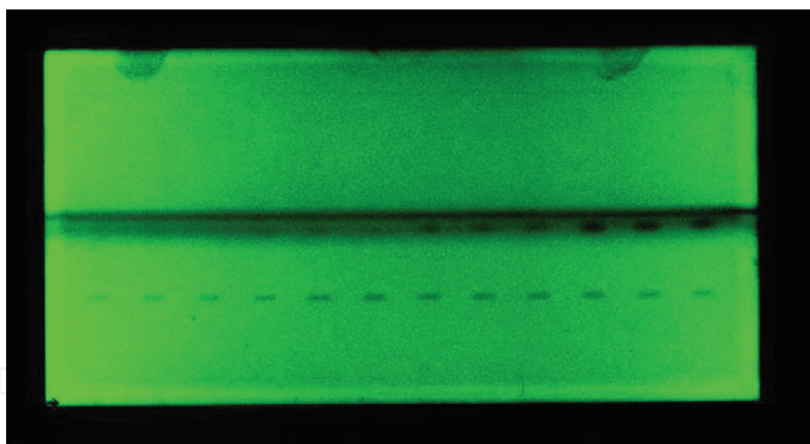


Figure 8.

Picture (video store, CAMAG) of plate at selectivity with parabens. Tracks 1–3, fluphenazine hydrochloride; tracks 4–6, fluphenazine hydrochloride + methylparaben; tracks 7–9, fluphenazine hydrochloride + propylparaben; and tracks 10–12, fluphenazine hydrochloride + methylparaben + propylparaben. Bands of parabens can be observed at the solvent front.

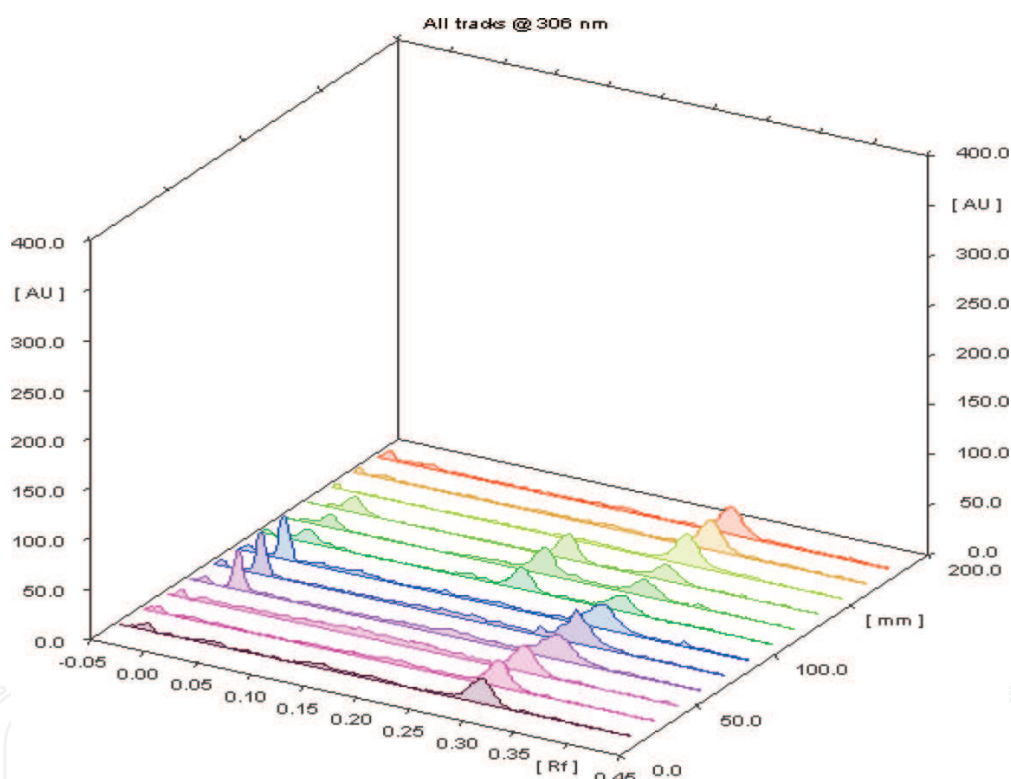


Figure 9.

Degradation study of fluphenazine with HCl, with NaOH, and with H_2O_2 . Tracks 1–3 (from bottom side to the upper side), fluphenazine; tracks 4–6, fluphenazine + HCl; tracks 7–9, fluphenazine + NaOH; and tracks 10–12, fluphenazine + H_2O_2 . R_f peak fluphenazine = 0.30. Other peaks at tracks 4–9: degradation products. R_f , retarding factor; AU, absorbance unit.

product. R_f for fluphenazine = 0.30 and R_f for degradation product = 0.56 (**Figure 10**) [38]. One of the products of photolysis mentioned in literature is a sulfoxide [2].

2.5 Stability-indicating HPLC method for quantification of risperidone in tablets

Risperidone is a benzisoxazole derivative (**Figure 11**), a second-generation anti-psychotic agent, that is chemically unrelated to other antipsychotic drugs. It is used for the treatment of schizophrenia, bipolar disorder, and irritability in children with autism. The major impurity products in the dosage forms, including degradation

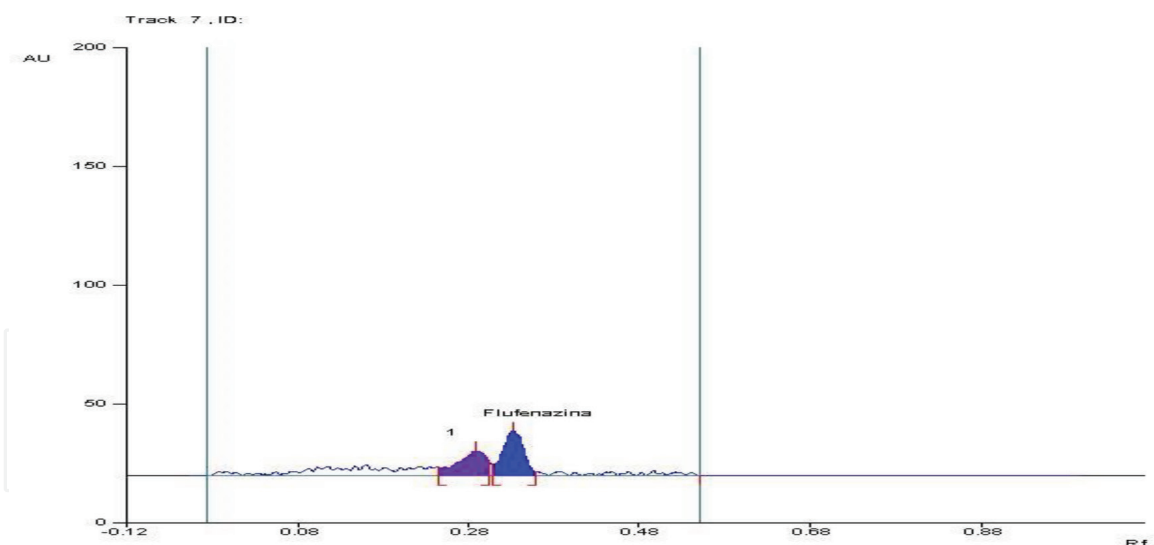


Figure 10.
 Degradation study of fluphenazine with VIS radiation. Peak no. 1 (from left to right), fluphenazine, and peak no. 2, degradation product. Rf, retarding factor; AU, absorbance unit.

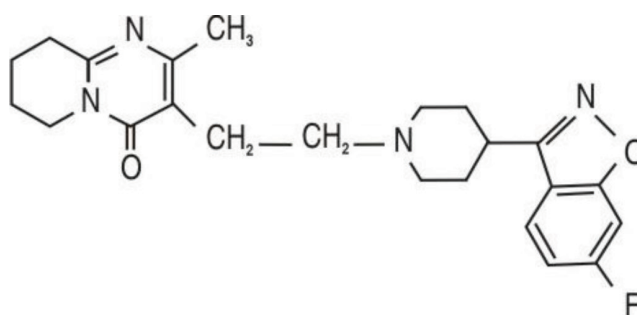


Figure 11.
 Chemical structure of risperidone.

products, described in USP are Z-oxime, bicyclo risperidone, and cis-risperidone N-oxide [3]. Some degradation products found in work investigations had been 9-hydroxyrisperidone and N-oxide of risperidone [32–35].

A stability-indicating LC method was developed and validated for the determination of risperidone in tablets. Quantitation was achieved by LC/DAD at 294 nm over the concentration range of 25.00–250.00 µg/mL. Mobile phase was a mixture of water, glacial acetic acid 0.5%, triethylamine 0.8%, and acetonitrile (65.00, 0.32, 0.52, 34.16, v/v), using a Purospher STAR RP-18e 250 × 4.5 mm (5 µ) column (Merck KGaA, Darmstadt, Germany) and paroxetine as internal standard. The method exhibited an adequate linearity, with a correlation coefficient of 0.999, selectivity, precision (RSD ≤ 0.847%), and accuracy (recoveries from 99.55 to 101.35%).

Risperidone was subjected to the stress conditions of oxidative, acid, base, thermal, and photolytic degradation. Risperidone was found no degrade in basic or acid stress conditions, neither in thermal stress exposition (50, 70, and 100°C) nor at visible or UV stress conditions, during the time of the study. Only two degradation products were observed with peroxide oxidation, well resolved from analyte peak, proving the stability-indicating power of the method. The drug was highly labile to hydrogen peroxide (3%) at room temperature. After 6 hours, steep fall in the drug peak area was observed. Major degradation products appeared at tR = 2.0 and tR = 5.3. These peaks were resolved from risperidone (tR = 3.5) (**Figure 12**). Risperidone was degraded to 35.00% when it was exposed to room light for 8 days, and it was degraded

to 17.00% when it was exposed to 80°C for 6 hours (**Figure 13**) [39]. One degradation product found in work investigations had been N-oxide of risperidone [3, 32].

2.6 Stability HPLC methods for stress testing studies of quinapril/hydrochlorothiazide and candesartan/hydrochlorothiazide

Quinapril hydrochloride (QUIN), hydrochlorothiazide (HCTZ), and candesartan cilexetil (CAN) are drugs that are widely used in the management of a highly prevalent disease such as hypertension. They are used alone or as combination therapy; the association of QUIN/HCTZ and CAN/HCTZ is used as combination therapy in the treatment of patients whose blood pressure is not adequately controlled with any of the substances alone. QUIN (**Figure 14**) is an angiotensin-converting enzyme (ACE) inhibitor used alone or in combination with other classes of antihypertensive agents; it is a prodrug and has little pharmacological activity until hydrolyzed in the liver to quinaprilat [32, 34]. HCTZ (**Figure 15**) is a thiazide diuretic and antihypertensive agent [32], and CAN (**Figure 16**) is an angiotensin II receptor antagonist (AT₁) used alone or in combination with other classes of antihypertensive agents; CAN is a prodrug and has little pharmacological activity until hydrolyzed during absorption in the gastrointestinal tract to candesartan [32, 34]. All three drugs have chemical structures susceptible to degradation; therefore, it is important to determine their stability.

In order to assess the chemical stability of a compound, it is very important to have the appropriate analytical method, i.e., they must be stability-indicating [3]. The main target while developing these methods was to have a single method for separation between QUIN/HCTZ or CAN/HCTZ and their degradation products. A stability-indicating LC methods with DAD and ELSD detection were developed by the authors for the simultaneous determination of CAN/HCTZ [40] and QUIN/HCTZ [41].

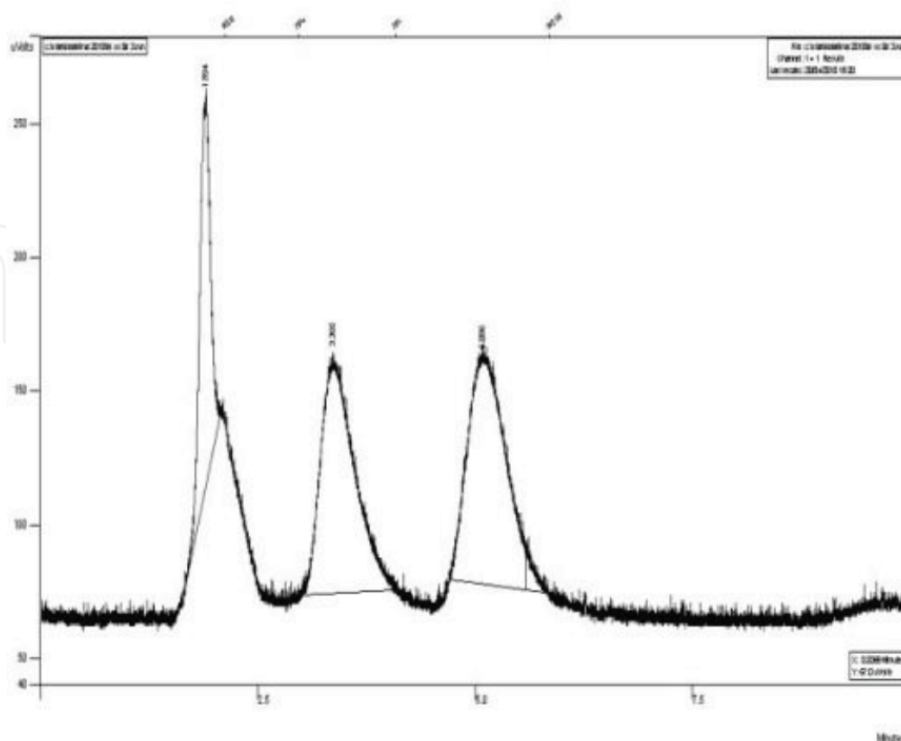


Figure 12.

Study at forced degradation of risperidone with 3% H₂O₂ at room light for 8 days and with 3% H₂O₂ at 80°C for 6 h. Peak 1 (t_R = 2.0): degradation product. Peak 2 (t_R = 3.5): risperidone. Peak 3 (t_R = 5.3): degradation product.

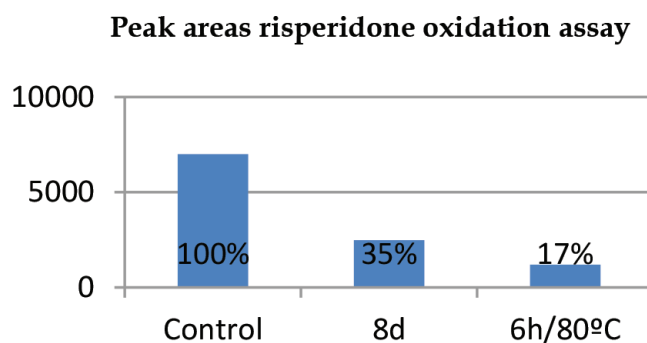


Figure 13.
Risperidone degradation at 3% H₂O₂ exposition.

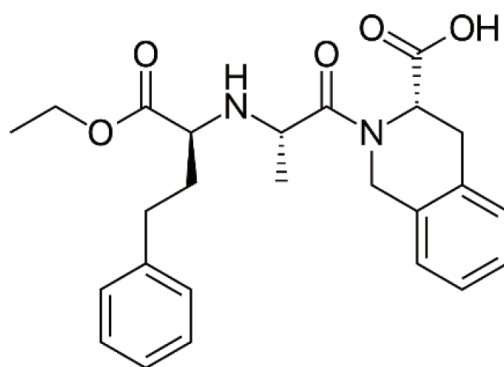


Figure 14.
Chemical structure of quinapril.

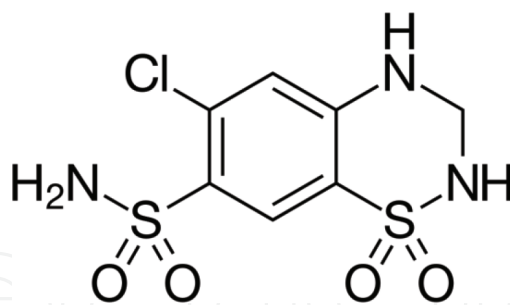


Figure 15.
Chemical structure of hydrochlorothiazide.

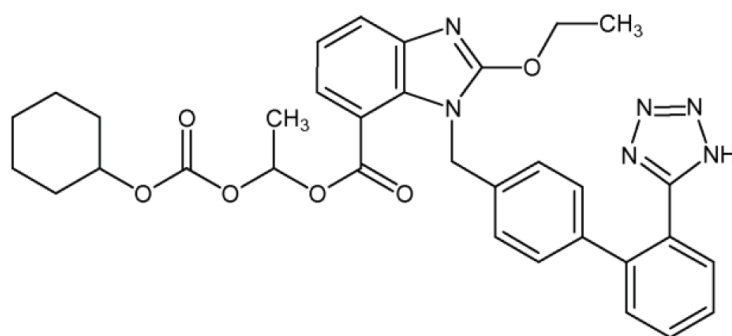


Figure 16.
Chemical structure of candesartan.

2.6.1 CAN/HCTZ

HPLC analyses were carried out on a Purospher® RP-18 column (125 mm × 4 mm, 5 µm; Merck KGaA, Darmstadt, Germany). Valsartan was used as internal standard (IS) at 70.0 µg min⁻¹. For LC/DAD method, the mobile phase consisted of acetonitrile (A) and phosphate buffer (pH 6.0; 0.05 M) (B) in a gradient mode. The flow rate was set to 1 mL min⁻¹, with UV detector wavelength fixed at 225 nm, and the column temperature was set at 30°C. For LC/ELSD method, the mobile phase consisted of acetonitrile (A) and water with acetic acid (0.175 M) and triethylamine (0.06 M) (pH 4.1) (B) in a gradient mode. The flow rate was set to 0.8 mL min⁻¹ and the column temperature was set at 35°C. ELSD evaporation temperature was set at 40°C, the gain was 7, and the nebulizer gas pressure was kept at 3 bar. The response with ELSD was fitted to a power function and the DAD response by a linear model over a range of 32–160 µg/mL for CAN and 25–125 µg/mL for HCTZ. The precision and accuracy of the methods were similar, with RSD below 3.0% and recovery between 98.1 and 103.9%. The drugs were subjected to stress conditions of hydrolysis, oxidation, photolysis, humidity, and temperature. Both drugs were mainly degraded by hydrolysis, showing the formation of one degradation product for HCTZ identified by MS/MS as 4-amino-6-chloro-1,3-benzendisulfonamide (DSA) and two for CAN cilexetil identified as candesartan and desethyl candesartan cilexetil. The degradation products were satisfactory separated from the main peaks and from each other as shown in **Figure 17**.

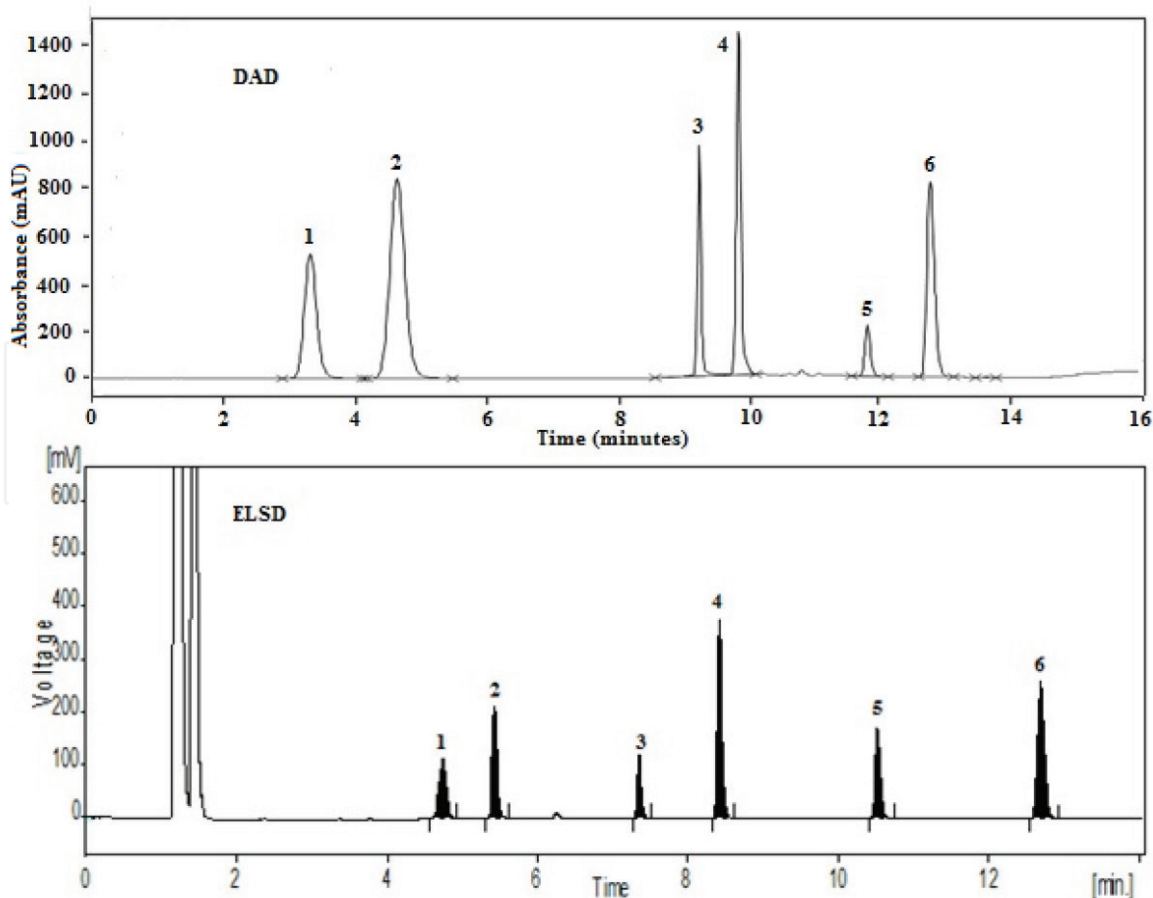


Figure 17.

(1) degradation product of HCTZ (DSA), (2) HCTZ, (3) alkaline degradation product of CAN (candesartan), (4) IS (valsartan), (5) acidic and neutral degradation product of CAN (desethyl candesartan cilexetil), and (6) CAN.

2.6.2 QUIN/HCTZ

HPLC analyses were carried out on a Chromolith[®] High Resolution RP-18 column (100 mm × 4.6 mm). For LC/DAD method, the mobile phase consisted of acetonitrile (A) and phosphate buffer (pH 3.0; 0.01 M) (B) in a gradient mode. The flow rate was set to 1.5 mL min⁻¹ with UV detector wavelength fixed at 215 nm, and the column temperature was set at 30°C. For LC/ELSD method, the mobile phase consisted of acetonitrile (A) and water with acetic acid (0.086 M) and triethylamine (0.007 M) (pH 3.3) (B) in a gradient mode. The flow rate was set to 1.0 mL min⁻¹, and the column temperature was set a 35°C. ELSD evaporation temperature was set at 40°C, the gain was 7, and the nebulizer gas pressure was kept at 3 bar. The analytes were eluted within 7 minutes in both methods. The response

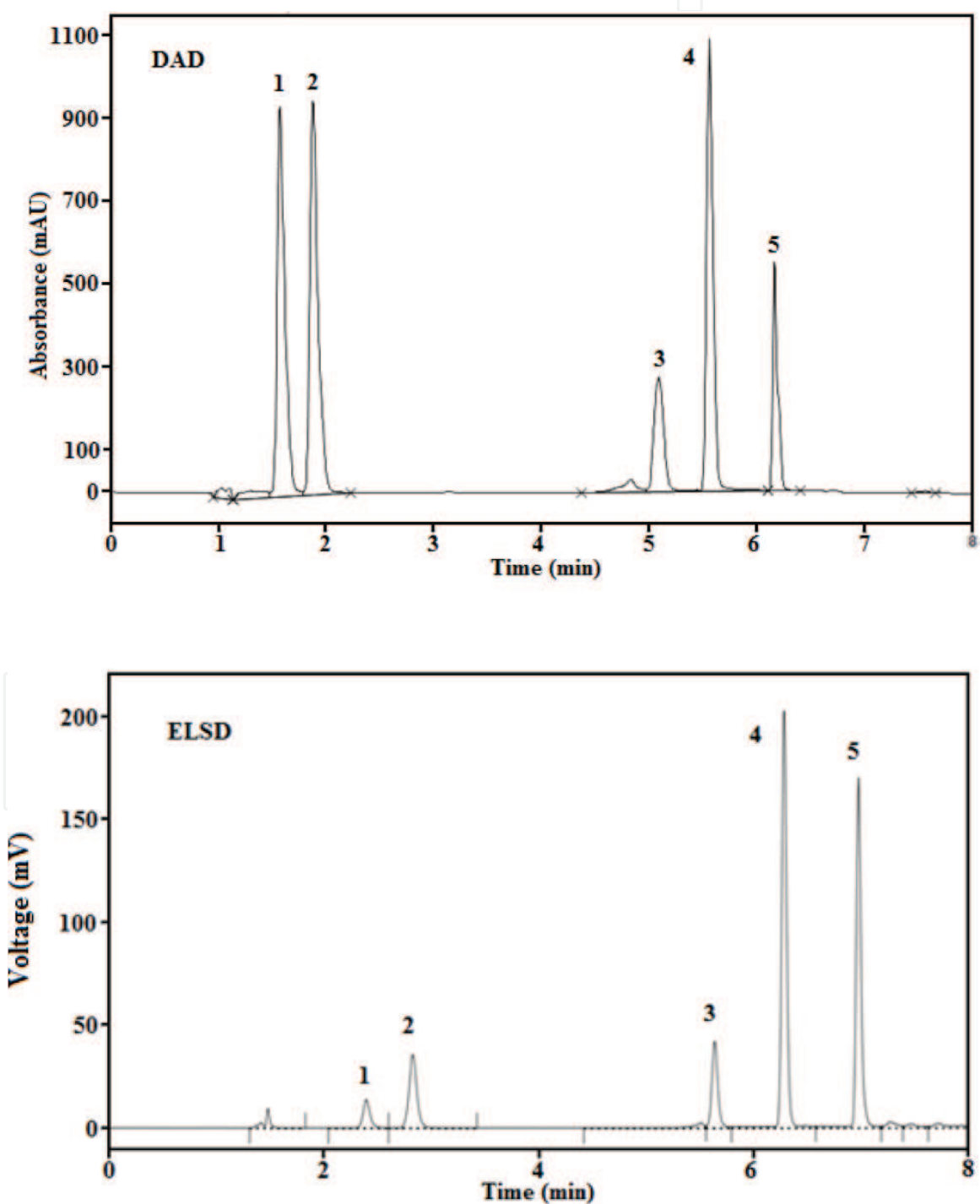


Figure 18.
Chromatogram with DAD and ELSD: (1) DSA, (2) HCTZ, (3) quinaprilat, (4) QUIN, and (5) DKP.

with DAD was linear, and the response with ELSD was fitted to a power function, for quinapril and hydrochlorothiazide concentrations of 20–160 $\mu\text{g mL}^{-1}$ and 12.5–100 $\mu\text{g mL}^{-1}$, respectively. DAD method achieved better precision than ELSD method, the LOQ of DAD was lower, and the accuracy of the methods was similar. Quinapril was subjected to hydrolytic, oxidative, thermal, humidity, and photolytic stress conditions. Quinapril was degraded by hydrolysis and thermal stress, showing the formation of quinaprilat and quinapril diketopiperazine as degradants, which were identified by MS/MS. Degradation products were well resolved from the main peaks and from each other, proving the stability-indicating power of the methods as shown in **Figure 18**.

2.7 Stability-indicating HPLC method for quantification of vortioxetine in bulk and tablets

Vortioxetine hydrobromide (**Figure 19**) is a serotonergic novel antidepressant with multiple pharmacologic activities [32, 41], approved by the US Food and Drug Administration in 2013 [42] for the treatment of major depressive disorder in adults. It is a phenylpiperazine derivative. Although the precise mechanism of action is not fully understood, it is thought to be related to enhancement of serotonergic activity in the central nervous system through inhibition of serotonin reuptake [32, 42]. It has a chemical structure susceptible to degradation; therefore, it is important to determine its stability by suitable analytical methods.

A simple HPLC method with photodiode array detection (DAD) was developed and validated by the authors [43], for determination of VOR in bulk and tablets, in the presence of its major degradation products. A C-18 column was used, with mobile phase consisting of acetonitrile and water with acetic acid and triethylamine in isocratic elution mode, with detection at 228 nm and 1.0 mL/min flow rate. Under these conditions, all the analytes were eluted within 15 min. Bromazepam was used as internal standard at 25 $\mu\text{g/mL}$. The assay was linear in the 25–125 $\mu\text{g/mL}$ concentration range. For precision, the RSD was lower than 1.8%, the recovery was between 100.0 and 101.6%, and the method demonstrated adequate selectivity (**Figure 20**). The drug was subjected to oxidative, hydrolytic, and photolytic stress conditions, showing significant degradation under oxidation with complete degradation after 1 day of stress, and was stable under acid, alkaline, neutral, and photolytic conditions. One main oxidative degradation product was formed, which was identified by ESI-MS/MS as the benzylic alcohol of VOR (**Figure 21**).

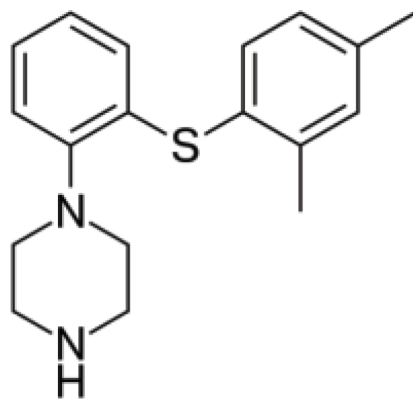


Figure 19.
Chemical structure of vortioxetine.

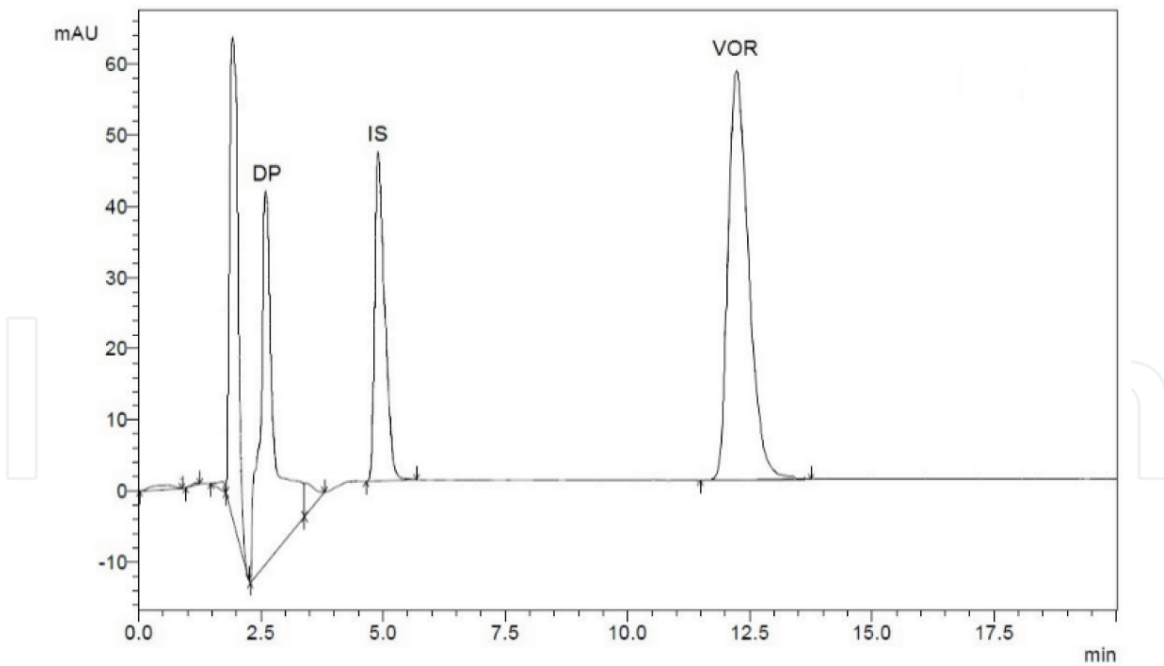


Figure 20.
Chromatogram of VOR, internal standard (IS), and oxidative degradation product (DP).

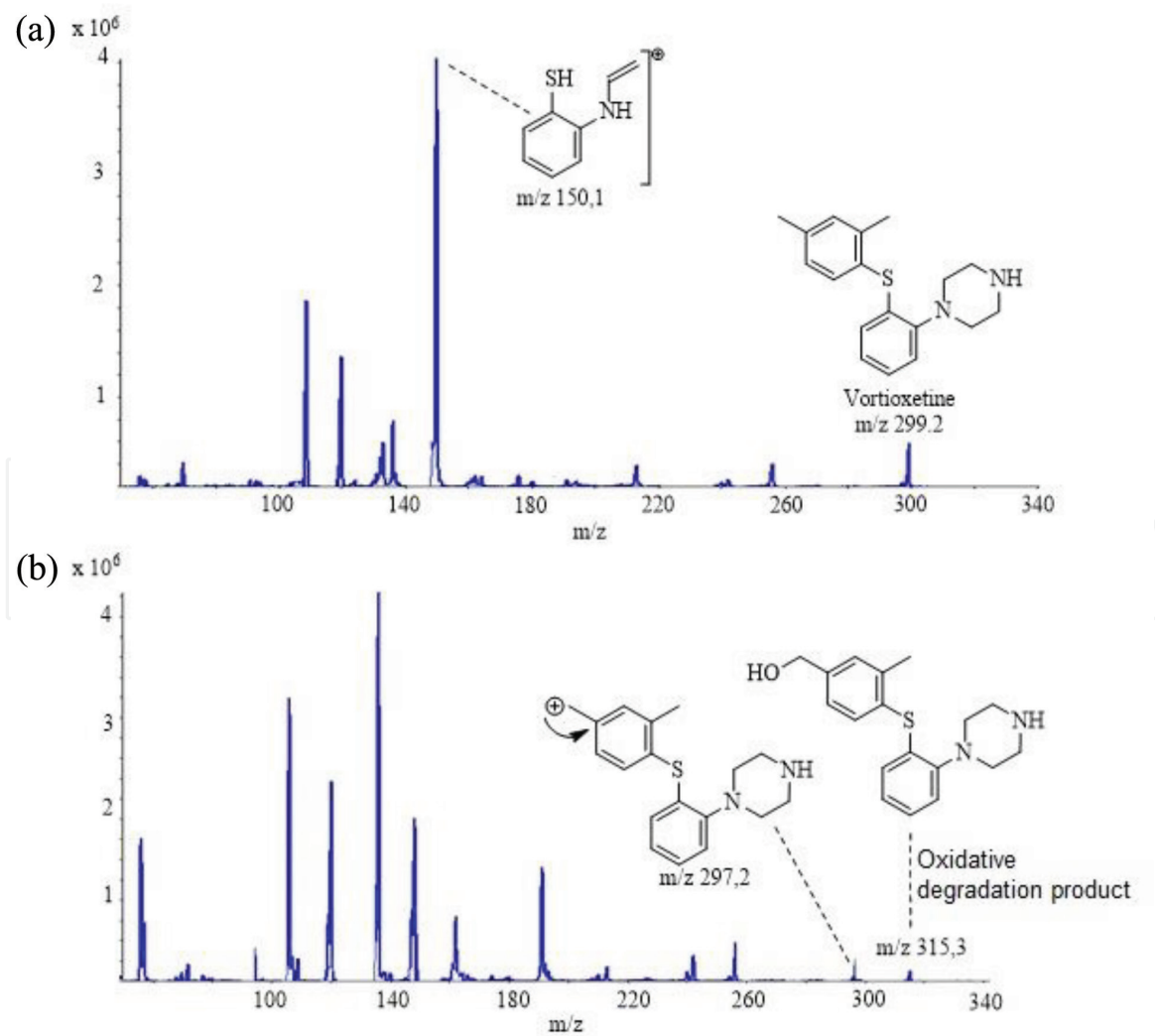


Figure 21.
The mass spectrum of vortioxetine standard (a) and oxidative degradation product (b).

3. Conclusions

The stability of a pharmaceutical is defined by its resistance to different chemical, physical, and microbiological reactions that may change their original properties. The stability of a pharmaceutical product is closely related to its potency; therefore, whether the compounds are degraded, a decrease of the therapeutic effect or changes in their toxicological properties can be produced, affecting their efficacy and safety, which becomes important to maintain a stable pharmaceutical product and to have the analytical tools to demonstrate stability. Therefore, stability-indicating methods are required to the quality control of pharmaceuticals.

The examples presented here are stability-indicating methods since they allow the determination of drugs in the presence of their degradation products, according to the International Conference on Harmonization (ICH) guidelines.

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Conflict of interest statement


Authors have no conflict of interest.

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